Docosahexaenoic Acid but Not Eicosapentaenoic Acid Lowers Ambulatory Blood Pressure and Heart Rate in Humans

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Abstract—Animal studies suggest that the 2 major ω3 fatty acids found in fish, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), may have differential effects on blood pressure (BP) and heart rate (HR). The aim of this study was to determine whether there were significant differences in the effects of purified EPA or DHA on ambulatory BP and HR in humans. In a double-blind, placebo-controlled trial of parallel design, 59 overweight, mildly hyperlipidemic men were randomized to 4 g/d of purified EPA, DHA, or olive oil (placebo) capsules and continued their usual diets for 6 weeks. Fifty-six subjects completed the study. Only DHA reduced 24-hour and daytime (awake) ambulatory BP ($P<0.05$). Relative to the placebo group, 24-hour BP fell 5.8/3.3 (systolic/diastolic) mm Hg and daytime BP fell 3.5/2.0 mm Hg with DHA. DHA also significantly reduced 24-hour, daytime, and nighttime (asleep) ambulatory HRs ($P=0.001$). Relative to the placebo group, DHA reduced 24-hour HR by 3.5±0.8 bpm, daytime HR by 3.7±1.2 bpm, and nighttime HR by 2.8±1.2. EPA had no significant effect on ambulatory BP or HR. Supplementation with EPA increased plasma phospholipid EPA from 1.66±0.07% to 9.83±0.06% ($P<0.0001$) but did not change DHA levels. Purified DHA capsules increased plasma phospholipid DHA levels from 4.00±0.27% to 10.93±0.62% ($P<0.0001$) and led to a small, nonsignificant increase in EPA (1.52±0.12% to 2.26±0.16%). Purified DHA but not EPA reduced ambulatory BP and HR in mildly hyperlipidemic men. The results of this study suggest that DHA is the principal ω3 fatty acid in fish and fish oils that is responsible for their BP- and HR-lowering effects in humans. These results have important implications for human nutrition and the food industry. **(*Hypertension*. 1999;34:253-260.)**

Key Words: eicosapentaenoic acid ■ docosahexaenoic acid ■ fatty acids ■ blood pressure ■ heart rate

Current evidence from epidemiological studies, clinical trials, and experimental animal studies suggests that ω3 fatty acids of marine origin may be protective against cardiovascular disease.[1] Most studies that assessed the potential cardiovascular benefits of ω3 fatty acids have focused largely on the importance of eicosapentaenoic acid (EPA), with little attention given to the relative effect of docosahexaenoic acid (DHA). This is probably attributable to the fact that the majority of commercial marine oil preparations as well as most, but not all, fish species contain more EPA than DHA. In addition, EPA, unlike DHA, is a substrate for the cyclooxygenase and lipoxygenase enzymes involved in eicosanoid metabolism.

Fish oil supplementation in humans results in substantial increases in plasma and tissue ω3 fatty acids, particularly EPA and DHA, but with variable incorporation in different phospholipid classes in different tissues. In vitro animal and human studies have shown that EPA and DHA are differentially incorporated into plasma,[2] platelet,[3,4] and tissue lipids.[4] These differences may play an important role in the utilization and metabolism of the 2 fatty acids. For example, EPA and DHA differ in their effects on membrane fluidity and the activities of membrane-bound enzymes[5] and on neutrophil-mediated endothelial detachment.[6] Compelling evidence shows that in vitro DHA but not EPA decreased cytokine-induced expression of endothelial leukocyte adhesion molecules.[7] Recent reports have described differences in lipid metabolism[8,9] and platelet aggregation.[10]

An antihypertensive effect of fish oils has been demonstrated in hypertensive patients,[11–14] although generally only when relatively large doses of fish oils have been used. We recently reported that daily fish meals that provide 3.65 g/d of ω3 fatty acids significantly reduced blood pressure (BP) in overweight, treated hypertensives.[15] This study addresses the question of whether EPA and DHA have differential effects on BP and heart rate (HR) in humans. In support of a differential effect of EPA and DHA on BP control, McLennan et al[16] recently reported that DHA was more effective than EPA at retarding the development of hypertension in spontaneously hypertensive rats (SHR) but not in adult SHR with already established hypertension. In addition, DHA but not EPA inhibited ischemia-induced cardiac arrhythmias at low dietary intakes in Hooded Wistar rats.[16] At

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moderate to high dietary intakes, DHA was also more effective than EPA at inhibiting thromboxane-like vasoconstrictor responses in the aortas from SHR.[16] Other studies have shown that dietary DHA prevented the development of hypertension in the stroke-prone SHR[17] and that EPA, compared with γ-linoleic acid, reduced the elevation of BP in the SHR without affecting HR.[18] In contrast, in the only human study to date, EPA and DHA supplementation in healthy, nonsmoking men failed to show any difference between the 2 fatty acids or any change in BP.[19]

Given the in vitro and in vivo data from animal studies that suggest that EPA and DHA may differ in their effects on BP and HR, we conducted a double-blind, randomized, placebo-controlled study to examine these possible differences in mildly hyperlipidemic men who are at increased risk of cardiovascular disease.

Methods

Study Population

Mildly hyperlipidemic (cholesterol ≥6 mmol/L and/or triglyceride ≥1.8 mmol/L) but otherwise healthy, nonsmoking men between the ages of 20 to 65 years were recruited from the general community by media advertising. Entry criteria included a body mass index between 25 and 30 kg/m^2, with no recent (previous 3 months) symptomatic heart disease, diabetes, or liver or renal disease (plasma creatinine >130 μmol/L); and not on regular nonsteroidal anti-inflammatory drug therapy, antihypertensive drugs, or lipid-lowering or other drugs that affect lipid metabolism. All subjects usually ate not >1 fish meal per week and drank <210 mL of ethanol per week. Fifty-nine of 136 subjects screened satisfied the entry criteria. The study was approved by the ethics committee of the Royal Perth Hospital, and all subjects gave written consent. All procedures performed were in accordance with institutional guidelines.

Dietary Education and Intervention

During a 3-week baseline period, subjects continued their usual diet and alcohol intake and, after collection of baseline measurements, were randomly assigned to 1 of 3 groups and matched for age and body mass index. Treatment groups were allocated to 4 g/d of EPA, DHA, or olive oil placebo capsules. Capsules that contained purified preparations of EPA ethyl ester (≈96%), DHA ethyl ester (≈92%), or olive oil (≈75% oleic acid ethyl ester) were provided by the Fish Oil Test Materials Program and the US National Institutes of Health and Department of Commerce. During the 6 weeks of intervention, all volunteers were asked to maintain their usual diets, alcohol intake, and physical activity and not to alter their lifestyle.

At an initial interview with a dietitian, subjects were given written and oral instructions on how to keep diet records, with food weighed or measured. Dietary intake was monitored by the same dietitian throughout the study, with completion of a 3-day diet record (2 weekdays and one weekend day) at baseline and repeated at the end of the 6-week intervention. Volunteers were also seen at 2-week intervals by the dietitian, who determined whether their usual eating habits were maintained and reminded them to make no changes.

Urinary Analytes, Lifestyle Assessment, and Anthropometry

Twenty-four–hour urinary sodium, potassium, calcium, and creatinine were measured at baseline and at the end of the intervention. Alcohol intake, physical activity, and use of medications were monitored every second week during the intervention with 7-day retrospective diaries. Weight was measured on an electronic scale; subjects wore light clothing and did not wear shoes. Height was measured with a stadiometer.

TABLE 1. Baseline Characteristics of Participants

	Olive Oil (n=20)	EPA (n=19)	DHA (n=17)
Age, y	48.4±2.0	48.9±1.7	49.1±2.2
BMI, kg/m^2	28.4±0.5	29.0±0.7	28.9±0.7
Waist-to-hip ratio	0.94±0.01	0.93±0.01	0.94±0.01
Serum cholesterol, mmol/L	6.47±0.21	6.20±0.20	6.18±0.18
Serum triglycerides, mmol/L	2.04±0.19	2.01±0.19	2.25±0.40
Supine systolic BP, mm Hg*	119.1±2.3	120.6±2.6	124.2±3.1
Supine diastolic BP, mm Hg*	71.4±1.4	73.6±1.8	75.2±1.6
Supine heart rate, bpm*	64.1±1.9	66.1±1.7	65.5±2.1

Values are expressed as mean±SEM.

*Average from 20 readings on 2 separate days in the clinic with a Dinamap 1846 SX/P monitor. Baseline measures were compared by 1-way ANOVA and were not significantly different.

Ambulatory BP Monitoring

Ambulatory BP (ABP) was monitored over 24 hours at baseline and at the end of intervention with an ambulatory blood pressure monitoring system (Accutracker II, Suntech Model 104) fitted by a trained nurse who instructed the subject on its use. The recorder was preset to record BP and HR every 30 minutes during waking hours (daytime) and hourly during sleep (nighttime). BP records were not visible to the subjects. Volunteers completed a diary that indicated their activity at the time of the ABP reading. When the monitoring system detected an error in BP measurement, subjects were instructed to rectify the error or return to the department to have the recorder corrected. After readjustment of the recorder, a BP reading was initiated to check correct functioning. Readings associated with a test code and those with a difference of <20 mm Hg between systolic blood pressure (SBP) and diastolic blood pressure (DBP) were excluded from analysis.

Plasma Phospholipid Fatty Acids

Total ω3 fatty acids measured in plasma phospholipids included 20:5, 22:5, and 22:6, and ω6 fatty acids included 20:3, 20:4, and 22:4. Fatty acid analysis of plasma phospholipids was determined according to previously described procedures.[20]

Statistical Analysis

Diet records were analyzed with the use of a nutrition analysis software package (Diet 1 version 4, Xyris software) based on the NUTTAB database of Australian foods (1995A).[21] Data were analyzed by SPSS (SPSS Inc) with general linear model analysis to assess the effects of EPA and DHA. Significance levels were adjusted for multiple comparisons by the Bonferroni method. Values are mean±SEM.

Results

Study Population

Fifty-six of 59 subjects randomized completed the study. Those who withdrew were either unable to maintain the schedule of laboratory visits or comply with the capsules; 1 subject withdrew because of gastrointestinal symptoms. Baseline characteristics of the 3 groups are shown in Table 1 and confirmed that they were well matched for the entry criteria. Clinic SBP and DBP were higher in the DHA group but not significantly different from the control and EPA groups (supine SBP, $P=0.324$; supine DBP, $P=0.251$). No significant differences existed between the groups for any of the other variables at baseline.

TABLE 2. Body Weight, 24-h Urinary Sodium, Potassium, and Creatinine Excretion at Baseline and Postintervention

	Olive Oil (n=20)	EPA (n=19)	DHA (n=17)
Body weight, kg*			
Baseline	88.7±2.0	89.1±2.3	90.8±2.8
Postintervention	88.9±2.1	89.3±2.3	91.1±2.9
Urinary Na^+, mmol/24 h			
Baseline	151.3±10.6	187.3±18.6	189.5±19.9
Postintervention	209.2±16.2	209.8±15.9	182.5±14.9
Urinary K^+, mmol/24 h			
Baseline	78.5±4.0	83.2±4.9	84.1±5.3
Postintervention	84.1±4.3	89.0±6.1	80.1±5.2
Urinary creatinine, mmol/24 h			
Baseline	14.3±0.6	14.8±0.5	15.5±0.7
Postintervention	14.2±0.6	15.8±0.9	14.5±0.6
Urinary Na^+/K^+			
Baseline	2.0±0.1	2.3±0.3	2.3±0.2
Postintervention	2.7±0.2	2.3±0.2	2.4±0.3
Urinary Na^+/Creatinine			
Baseline	10.8±0.8	12.7±1.1	12.2±1.2
Postintervention	15.2±1.2	13.4±0.9	12.1±1.3

Values are expressed as mean±SEM. Baseline measures were compared by 1-way ANOVA and were not significantly different between groups. With general linear model analysis, there were no significant treatment effects for postintervention urinary sodium, potassium, creatinine, Na^+/K^+ ratio, and Na^+/creatinine ratio after adjustment for age, baseline weight, and baseline value.

*Postintervention body weight was analyzed after adjustment for age and baseline value.

Energy and Macronutrient Intake

No significant difference in body weight existed between the groups at baseline, and there was no significant change in weight during intervention in the 3 groups (Table 2). Analysis of diet records confirmed that total energy intake was not different at baseline between groups and remained unchanged during the 6 weeks of the intervention (Table 3). Similarly, there were no significant differences between the groups in any of the dietary nutrients at baseline, nor were there any significant changes during the intervention (Table 3). Alcohol consumption and physical activity remained unchanged during the intervention in all groups.

Plasma Phospholipid Fatty Acids

At baseline, there were no significant differences between groups in plasma phospholipid fatty acids, including EPA and DHA content. The changes in EPA and DHA from baseline to the end of intervention indicated compliance with capsule intake. After supplementation with purified EPA, plasma phospholipid 18:2ω6 decreased (20.92±0.47% to 16.00±0.52%, $P<0.0001$), 20:4ω6 decreased (12.18±0.43% to 9.34±0.32%, $P<0.0001$), and EPA increased from 1.66±0.07% to 9.83±0.06% ($P<0.0001$). At the same time, DHA composition remained relatively unchanged (4.11±0.24% to 4.02±0.13%, $P>0.05$). Supplementation with purified DHA increased the phospholipid composition of DHA from 4.00±0.27% to 10.93±0.62% ($P<0.0001$) and also led to a small, nonsignificant ($P=0.383$) increase in the EPA content (1.52±0.12% to 2.26±0.16%). Both 18:2ω6 (21.04±0.49% to 18.22±0.62%, $P<0.0001$) and 20:4ω6 (11.25±0.42% to 8.82±0.32%, $P<0.0001$) were reduced. Olive oil supplementation did not alter 18:2ω6, 20:4ω6, EPA, or DHA composition of plasma phospholipids.

Urinary Electrolytes

For all subjects combined, the mean 24-hour urinary sodium and potassium excretion values at baseline were 175.1±9.6 and 81.8±2.7 mmol/24 hours, respectively (Table 2). This was not significantly different between groups. With general linear model analysis, no significant treatment effects existed for postintervention urinary sodium after adjustment for age, baseline weight, and baseline value. Urinary potassium and creatinine excretion and the sodium/potassium and sodium/creatinine ratios were not significantly different between groups at baseline and at completion of the study (Table 2).

Ambulatory Blood Pressure

The mean values for SBP and DBP at baseline and postintervention during the 24 hours of ABP monitoring for each group are shown in Figure 1. Mean 24-hour, daytime, and nighttime ABP by group are shown in Table 4. Although baseline mean 24-hour BP was higher in the DHA group by ≈5/3 mm Hg (SBP/DBP), this was not significantly different from the control and EPA groups (SBP, $P=0.257$; DBP, $P=0.189$). Supplementation with DHA significantly reduced blood pressure. In general linear model analysis, with mean 24-hour BP as the dependent variable, DHA significantly effected the reduction of the SBP (−5.8±2.1 mm Hg, $P=0.022$) and DBP (−3.3±1.3 mm Hg, $P=0.029$) compared with the olive oil placebo after adjustment for age, baseline weight, and baseline BP. There was also a significant effect of DHA on mean daytime SBP (−3.5±2.9 mm Hg, $P=0.041$) and DBP (−2.0±1.1 mm Hg, $P=0.046$) compared with the placebo group after adjustment for age, baseline weight, and baseline value. There was no significant effect of DHA on mean nighttime SBP and DBP. The results remained significant in models that adjusted for changes in urinary sodium, potassium, or creatinine or the sodium/potassium ratio. There was no significant effect of EPA on 24-hour, daytime, or nighttime BP.

Heart Rate

Hourly mean values for HR at baseline and postintervention during the 24 hours of ABP monitoring for each group are shown in Figure 1. Mean 24-hour, daytime, and nighttime HRs by treatment group are shown in Table 4. Relative to controls, there was a significant effect of DHA on 24-hour (−3.5±0.8 bpm, $P=0.001$), daytime (−3.7±1.2 bpm, $P=0.001$), and nighttime (−2.8±1.2 bpm, $P=0.025$) ambulatory HRs after adjustment for age, baseline weight, and baseline value. EPA had no significant effect on 24-hour ambulatory HR.

Discussion

This study addressed the question as to whether highly purified EPA or DHA administered in the diet differ from one

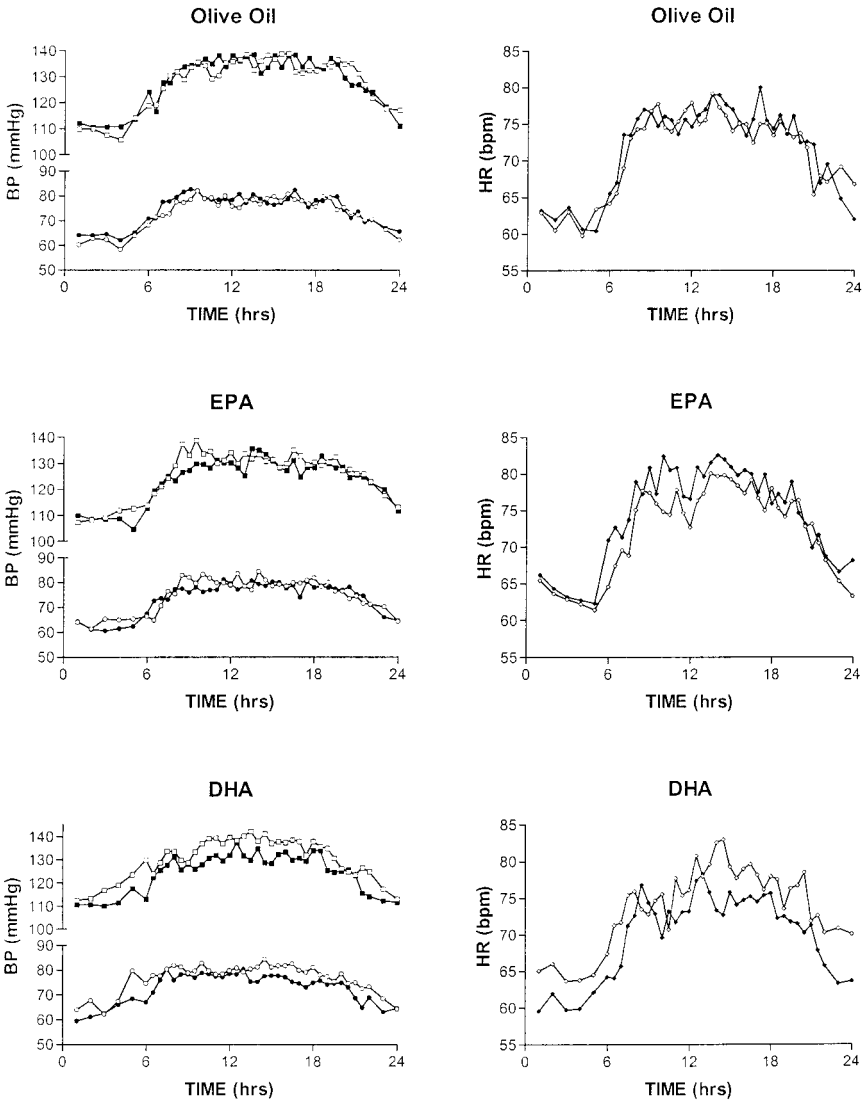
TABLE 3. Total Energy Intake and Macronutrients at Baseline and Postintervention

	Olive Oil (n=20)	EPA (n=19)	DHA (n=17)	ANOVA at Baseline (*P*) Treatment Effects (*P*)
Total energy intake, kJ/d				
Baseline	10 441±588	9516±677	10 550±588	NS
Postintervention	9970±704	9652±844	9917±613	NS
Total fat, % of energy				
Baseline	34.2±1.2	30.9±1.6	32.6±1.6	NS
Postintervention	33.7±1.1	33.5±1.7	35.2±1.6	NS
Saturated fat, % of energy				
Baseline	13.6±0.8	12.1±0.9	13.6±0.9	NS
Postintervention	13.6±0.9	13.3±1.0	14.4±1.1	NS
Monounsaturated fat, % of energy				
Baseline	12.2±0.6	11.1±0.8	11.5±0.7	NS
Postintervention	12.0±0.4	12.3±0.8	12.1±0.6	NS
Polyunsaturated fat, % of energy				
Baseline	5.0±0.3	4.2±0.3	4.6±0.2	NS
Postintervention	4.9±0.4	4.6±0.4	5.5±0.5	NS
Protein, % of energy				
Baseline	18.0±0.5	19.8±0.9	18.1±0.6	NS
Postintervention	17.4±0.6	19.0±0.9	18.0±0.7	NS
Carbohydrate, % of energy				
Baseline	42.1±1.7	44.6±1.7	41.8±1.8	NS
Postintervention	44.0±1.2	43.7±1.9	40.5±1.7	NS
Fiber, g/d				
Baseline	30.8±2.9	27.4±1.8	26.1±1.1	NS
Postintervention	27.5±3.1	24.9±2.2	25.4±1.4	NS
Sodium, g/d				
Baseline	3.5±0.2	3.2±0.3	3.4±0.2	NS
Postintervention	3.1±0.3	3.5±0.3	3.4±0.3	NS
Potassium, g/d				
Baseline	3.7±0.2	3.5±0.2	3.6±0.3	NS
Postintervention	3.5±0.2	3.2±0.3	3.4±0.2	NS
Alcohol intake, g/wk				
Baseline	107.4±20.3	98.8±11.4	99.6±14.0	NS
Postintervention	100.3±11.8	86.7±6.2	93.9±11.5	NS

Values are expressed as mean±SEM. Baseline measures were compared by 1-way ANOVA. General linear model analysis was used to test for treatment effects on postintervention values adjusted for baseline value.

another with respect to effects on ABP and HR in humans. The study was performed against a background diet that was carefully standardized for the amount and type of dietary fats in overweight, mildly hyperlipidemic but otherwise healthy men. We found that purified DHA, but not EPA, resulted in a significant reduction in ABP and HR compared with placebo.

Interestingly, the BP-lowering effects of DHA were observed in men with otherwise normal BP. Their mean clinic BP at baseline was 121.2±1.5 mm Hg systolic and 73.3±0.9 mm Hg diastolic. To date, the most impressive results with respect to ω3 fatty acid effects on BP control have been observed in hypertensive patients.[11–14] Moreover, the antihypertensive effect in the present study was seen with 4 g/d of DHA, which is at the low end of the range of ω3 fatty acid intake. Previous trials have suggested that beneficial effects on BP were attainable only with relatively large doses of ω3 fatty acids.[22,23] We recently reported a significant reduction in ABP, particularly in daytime pressures and in overweight, treated hypertensive patients.[15] Patients who ate a daily fish meal that provided 3.65 g of ω3 fatty acids showed a BP fall of 6.8±2.6/5.1±1.7 mm Hg (systolic/diastolic). This finding differs from that reported by Grimsgaard et al,[19] in which neither 4 g/d of EPA nor DHA altered clinical BP in healthy, nonsmoking men. The mean clinical BP at baseline in the study by Grimsgaard et al (122/77 mm Hg) was similar to that of the subjects used in our study. Reasons for the discrepancy between the studies may relate to differences in the background diets or to the improved statistical power in showing a reduction in BP



Twenty-four–hour ambulatory mean SBP, DBP, and HR at baseline and postintervention in the 3 groups: □ indicates baseline SBP; ■, postintervention SBP; ○, baseline DBP; ●, postintervention DBP; ◇, baseline HR; and ◆, postintervention HR.

through the use of ABP monitoring. The technique enables continuous recording of BP every 30 minutes during awake periods and hourly when subjects are asleep. Our subjects were older (≈5 years), although it is unlikely that this would have affected the outcome.

There is support in the literature for DHA being the principal active ω3 fatty acid of fish and fish oils involved in lowering BP, but to date this has been demonstrated in animals only.[16,17] Kimura et al[17] showed that DHA, compared with a diet free of DHA, prevented the development of hypertension in stroke-prone SHRs by inhibiting the increase in SBP in a dose-dependent manner. McLennan et al[16] compared EPA with DHA in rats and related the changes to olive oil. DHA retarded the development of high BP when fed to young prehypertensive SHRs. EPA also retarded the development of hypertension, but less so than DHA. Interestingly, neither DHA nor EPA modified BP in the adult SHR with established hypertension. It was found that in the adult SHR, DHA but not EPA reduced the vascular thromboxane-like vasoconstrictor responses in aortas after inhibition of nitric oxide with N^{ω}-nitro-L-arginine. In contrast, Sasaki et al[18] showed that EPA, compared with γ-linoleic acid, significantly reduced the rise in BP in 11-week-old SHRs.

With animal models, we and others have shown that the antihypertensive effect of ω3 fatty acids may be related to improved endothelial vasodilator function,[24,25] reduced pressor reactivity of resistance vessels,[25,26] and increased vascular compliance.[27] We also produced evidence that the increased endothelial relaxant effects were in part due to suppression of thromboxane A_2 or cyclic endoperoxides, with the possible additional effect of enhanced endothelial nitric oxide synthesis.[25] Other investigators[28–30] have shown that the consumption of fish oil in humans leads to reduced forearm vascular reactivity to angiotensin II and norepinephrine. In addition, it was possible to antagonize the blunting effect of fish oils in response to both norepinephrine and angiotensin II in human forearm resistance arteries by oral administration of indomethacin, which suggests that ω3 fatty acids exert their suppressive effects partially by modification of the prostanoid products derived from the cyclooxygenase pathway.[31] The mechanisms by which BP is reduced to a greater extent by

TABLE 4. Mean 24-h, Daytime, and Nighttime BP and HR at Baseline and Postintervention

	Olive Oil (n=20)	EPA (n=19)	DHA (n=17)	ANOVA at Baseline (*P*) Treatment Effect (*P*) EPA	DHA
Mean 24-h SBP, mm Hg					
Baseline	123.0±3.4	122.1±2.2	127.8±3.7	NS	
Postintervention	124.0±3.0	119.6±2.3	122.0±3.4	−2.5±1.6	−5.8±2.1
				NS	(0.022)
Mean 24-h DBP, mm Hg					
Baseline	70.0±1.2	71.9±1.6	73.6±2.0	NS	
Postintervention	71.4±1.1	70.6±1.3	70.3±1.6	−1.3±1.1	−3.3±1.3
				NS	(0.029)
Mean 24-h HR, bpm					
Baseline	69.0±2.0	69.6±1.4	71.5±1.7	NS	
Postintervention	69.8±1.8	71.6±1.3	68.0±1.5	2.0±1.4	−3.5±0.8
				NS	(0.001)
Daytime SBP, mm Hg					
Baseline	133.1±3.6	131.4±2.6	137.7±4.2	NS	
Postintervention	133.5±2.8	130.8±2.2	134.2±3.5	−0.6±2.0	−3.5±2.9
				NS	(0.041)
Daytime DBP, mm Hg					
Baseline	76.6±1.2	78.0±1.6	79.5±2.0	NS	
Postintervention	78.0±1.3	77.9±1.5	76.6±1.8	−0.1±0.7	−2.0±1.1
				NS	(0.046)
Daytime HR, bpm					
Baseline	73.8±2.0	75.2±1.5	76.3±1.9	NS	
Postintervention	75.5±1.5	77.5±1.6	72.6±1.7	2.7±1.7	−3.7±1.2
				NS	(0.001)
Nighttime SBP, mm Hg					
Baseline	112.9±3.5	112.8±2.6	117.9±3.8	NS	
Postintervention	114.5±3.0	112.9±2.2	115.7±2.8	0.2±2.2	−2.2±2.1
				NS	NS
Nighttime DBP, mm Hg					
Baseline	63.4±1.6	65.8±1.8	67.7±2.1	NS	
Postintervention	64.3±1.4	65.5±2.0	66.1±2.0	−0.3±1.5	−1.6±1.6
				NS	NS
Nighttime HR, bpm					
Baseline	64.1±2.1	64.0±1.4	66.8±2.1	NS	
Postintervention	63.7±1.5	66.3±1.4	64.0±1.9	2.3±1.5	−2.8±1.2
				NS	(0.025)

Values are expressed as mean±SEM. Baseline measures were compared by 1-way ANOVA. General linear model analysis was used to test for treatment effects on postintervention values adjusted for age, baseline weight, and baseline value. BP and HR were recorded every 30 minutes during waking hours (daytime) and hourly during sleep (nighttime).

DHA than EPA are not established. McLennan et al[16] postulated that DHA may serve as a regulating lipid to prevent thromboxane-induced contraction and perhaps restore the vasoconstrictor/vasodilator balance after impairment of the nitric oxide–related processes that normally function. It has not yet been established whether DHA inhibits thromboxane synthetase or thromboxane A_2/prostaglandin H_2 receptor function.[32] In addition, Hashimoto et al[33] recently showed that rats fed DHA intragastrically had reduced plasma norepinephrine levels. Increased adenyl purines such as ATP, which is released spontaneously and in response to norepinephrine from segments of caudal artery, were significantly inversely associated with BP. It was speculated that DHA alters membrane fatty acid composition and may

accelerate ATP release from vascular endothelial cells, which, in conjunction with reduced plasma norepinephrine, may be responsible for a reduction in BP.[33]

Reductions in HR similar to those seen in the present study have been reported after the consumption of fish or fish oil supplements.[12,34] We have shown a decrease of 3.1±1.4 bpm (P=0.036) in 24-hour and 4.2±1.6 bpm (P=0.013) in daytime HR after daily consumption of fish for 16 weeks in overweight, treated hypertensives.[15] The reductions in mean 24-hour, daytime, and nighttime HR with DHA in the present study were significant and comparable to those reported previously.[15] Interestingly, EPA resulted in a small, nonsignificant, rise in HR. Therefore, our results on HR changes are in accordance with those of Grimsgaard et al,[19] in which HR was decreased by 2.2 bpm (P=0.006) with DHA and increased by 1.9 bpm (P=0.04) with EPA.

The reduction in HR by DHA suggests that there may be a significant cardiac component to the antihypertensive effect of DHA, which is possibly mediated by effects on autonomic nerve function or β-adrenoreceptor activity. Animal studies have demonstrated that ω3 fatty acids are incorporated into myocardial cells and have potent antiarrhythmic effects.[35] It has also been shown that DHA is the major ω3 fatty acid incorporated into myocardial membranes, even when animals have been fed fish oils in which EPA has predominated.[36] These findings suggest an important role for DHA in cardiac function. The antiarrhythmic effects of ω3 fatty acids are thought to be related to their ability to inhibit myocardial Ca^{2+} overload,[37] thromboxane production,[38] ischemic acidosis, and ischemic K^+ loss.[39] In addition, Kang et al[40] and Weylandt et al[41] suggested that free polyunsaturated fatty acids and not polyunsaturated fatty acids in phospholipids had an inhibitory effect on the electrical automaticity and excitability of the cardiac myocyte rather than reduction in cytosolic Ca^{2+}. McLennan et al[16] showed that DHA but not EPA prevented ischemia-induced cardiac arrhythmias in Hooded Wistar rats that were fed purified oils for 5 weeks. The authors noted that although both EPA and DHA were antiarrhythmic in isolated neonatal myocytes,[37] the failure of EPA to exhibit antiarrhythmic effects in their study may reflect a threshold dose effect. However, the precise cellular mechanism for a differential effect of DHA and EPA remains unclear.

The results of this study may help clarify the potentially protective mechanisms of dietary ω3 fatty acids against cardiovascular disease and, in particular, demonstrate differential effects of EPA and DHA on BP regulation and HR in humans. The findings may have an important effect on the choice of ω3 fatty acid supplements and on the relative use of EPA and DHA in food nutrition in the form of incorporation into animal feeds or foodstuffs. As increasingly concentrated forms of fish oils are becoming available, their use as "over-the-counter" dietary supplements has increased, and fish oil concentrates are authorized by drug authorities for treatment of patients with certain types of hypertriglyceridemia. Therefore, it is of both theoretical interest and practical importance to understand the relative cardiovascular effects of EPA and DHA and whether the more highly purified compounds have differential effects from the combinations found in many fish oil extracts and, more importantly, in dietary fish.

In conclusion, this study in mildly hyperlipidemic but otherwise healthy men has shown that DHA may be the principal ω3 fatty acid in fish and fish oils that lowers BP and HR in humans. This observation has important implications for human nutrition and the food industry for incorporation of ω3 fatty acids into the human food chain.

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